Loss of Heterozygosity at *PTEN* in Sporadic Colorectal Adenomas

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PTEN, a putative tumour suppressor gene, has been mapped to 10q23.3. Mutations or allelic loss at PTEN have been reported in malignant tumours such as glicblastoma, melanoma, breast, prostate, and colorectal carcinomas; germ-line PTEN mutations have also been reported in some juvenile polyposis families.
To investigate PTEN in sporadic colorectal adenomas, we examined allele loss (LOH) at D10S201, D10S215, D10S541 in microdissected tissues from 74 adenomas.
Fifty-seven cases were informative, with seven adenomas (12.3%) showing LOH at one or more loci. The frequencies of LOH in adenomas with mild, moderate and severe dysplasia were 6.3% (1/16), 12.0% (3/25) and 18.8% (3/16), respectively, showing that loss of PTEN may be involved in a subset of adenomas.
These figures are lower than the frequency of 35% reported in sporadic colorectal carcinomas, and generally lower than the accepted 20% threshold for a significant frequency of LOH. Thus, loss of PTEN would appear to be associated with carcinoma rather than adenoma.
This is consistent with observations in other tumour types, where PTEN mutation appears related to generally more advanced malignancies. Further work is needed to define the possible PTEN mutations in these positive LOH cases.

Infrequent expression of CD40 on colorectal cancer: association with advanced Dukes stage

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We have used immunohistochemistry to investigate the expression of CD40 on a series of 160 cases of histologically confirmed cancers of the colon and rectum. CD40 was expressed by a minority of tumours (15/160), most of which showed a uniform staining pattern throughout the tumour specimen. However, in some positive samples expression was either weak and/or localised to isolated regions within the tumour. CD40 expression was not seen in 23 tumours at Dukes A stage, but was expressed in 4/58 and 11/79 Dukes B and C tumours, respectively. This was also reflected in poorer survival for patients with CD40-expressing tumours, although this trend was not statistically significant. A proportion of these tumours were immunostained for HLA class II molecules. We found that expression of HLA Class II decreased, with more advanced Dukes stage (A; 12/17, B; 22/44, C; 11/29). This data suggests that CD40 expression in colorectal cancer may have a negative impact on tne outcome of patient survival and may implicate a role for CD40-mediated stimulated growth of these tumours.

BAG-1 EXPRESSION IN NORMAL TISSUES, COLORECTAL ADENOMAS AND COLORECTAL CARCINOMAS

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Apoptosis is regulated by a large number of oncoproteins. Bcl-2 protects against the development of apoptosis in a variety of normal and neoplastic tissues. The more recently described Bag-1 protein also suppresses apoptosis by a mechanism which is at least partly mediated via interaction with Bcl-2. In a previous study we demonstrated the differential expression of Bcl-2 in colonic crypt epithelium in a cytoplasmic distribution and the over-expression of Bcl-2 in colonic carcinomas and adenomas. In this study we studied a number of normal tissues and a series of colorectal adenomas and carcinomas for the expression of Bag-1 by immunohistochemistry using a polyclonal antibody. In normal skin immunoreactivity was present within most keratinocytes in a predominantly nuclear distribution with sparing of the granular layer. In the small intestine only occasional epithelial cells within the crypt were positive in a predominantly nuclear pattern. In the stomach positive staining was observed in a few mucus-secreting epithelial cells in the antrum again in a predominantly nuclear distribution but surface foveolar cells were negative. In the large intestine there was immunoreactivity of epithelial cells within the crypt and at the surface in both cytoplasmic and nuclear distributions. In most colonic adenomas and carcinomas there was strong nuclear staining; cytoplasmic immunoreactivity was also present in a stronger staining pattern than in adjacent non-neoplastic mucosa. These findings suggest a role for Bag-1 in colorectal neoplasia and are consistent with interaction with Bcl-2.

AVERAGE VOLUME OF MITOCHONDRION, NUCLEOLUS, HETEROCHROMATIN AND EUCHROMATIN IN HEXAMETHYLENE BISACETAMIDE (HMBA) INDUCED HUMAN COLONIC CARCINOMA CELL LINE (LOVO)

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The present study aims to determine the effect of HMBA on the mitochondrial and nuclear components of LOVO (CCL-229) using electron microscopical and stereological techniques. 2mM and 4mM of HMBA were included in the culture medium for a period of 7 days and then for a further 3 days HMBA-containing medium was omitted. Control flasks were never exposed to HMBA. The nuclear-cytoplasmic ratio, volume density of mitochondria ($V_{VM,C}$), nucleolus ($V_{VNC,N}$), heterochromatin ($V_{VH,N}$) and euchromatin ($V_{VE,N}$) were estimated using point counting. The nuclear volume (V_N) was estimated from point-sampled intercepts. From these data cytoplasmic volume (V_{CYT}), cell volume (V_{CELL}), average volume of mitochondrion per cell ($V_{M,C}$) and average volume of nucleolus ($V_{VNC,N}$), heterochromatin ($V_{H,N}$) and euchromatin ($V_{E,N}$) per nucleus were calculated. The general trend for the average volumes of the mitochonrion and nucleolus decreased and the heterochromatin increased in HMBA-treated groups when compared with the control. There was a decrease in $V_{VM,C}$ on day 3 to day 10 of the culture period with the control groups having the highest value when compared with the HMBA-treated ones. However, statistical significant differences were only detected on day 3 in the 4mM HMBA-treated group and on day 10 in both HMBA-treated groups. Similar reduction in the values of $V_{M,C}$ were observed except that the HMBA-treated groups had values higher than the control on day 10 but no significant difference was detected in all time points amongst the tested groups. Significant decrease in $V_{VNC,N}$ was observed on day 1 to day 5 in HMBA-treated groups. Similar trend was observed in $V_{NC,N}$ and significant difference was only observed on day 1 between HMBA-treated and control groups. No statistical significant difference was detected in $V_{NC,N}$, $V_{VH,N}$, $V_{H,N}$, $V_{VE,N}$ and $V_{E,N}$ between HMBA-treated and control groups from day 1 to day 10. We conclude that HMBA has no significant effect on the nuclear and cellular metabolic activity in the human colonic carcinoma cell line (LOVO).
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